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The Relationship between Hypertension and Central Serotonergic Nervous System Activity in Spontaneously Hypertensive Rats

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Abstract□Relationship between the maintenance of hypertension and central serotonergic nervous system activity in spontaneously hypertensive rats (SHR) was studied. Serotonin turnover-rates were measured in 5 brain areas as an index of serotonergic neuronal activity and compared at the ages of 14 weeks in two types of animals; (1) spontaneously hypertensive rats (SHR) (2) normotensive wistar kyoto rats (WKY). In 14-week old SHR, central serotonin turnover rate was significantly lower in telencephalon, hypothalamus/thalamus and midbrain than normotensive rat, but significantly higher in cerebellum. There were no significant differences between serotonin turnover rate in pons/medulla of SHR and that of normotensive rat. These data suggest that the abnormally lower turnover rates of serotonin in telencephalon, hypothalamus/thalamus and midbrain may be one of the underlying neuronal factors for manifestation of hypertension in SHR.

Keywords□SHR, Hypertension, CNS, serotonergic System, turnover rate.

In recent years evidence has been accumulated to indicate that central serotonergic neurons participate in the regulation of blood pressure[1]. Anatomically, areas of the brain stem and spinal cord involved in vasomotor control (i.e., the intermediolateral cell column, nucleus tractus solitarius and areas of the hypothalamus) are heavily innervated by serotonin-containing neurons[2]. Pharmacologic evidence also suggests that central serotonergic neurons participate in cardiovascular control[3]. Apart from a limited number of experiments which have attempted by indirect means to determine the role of serotonin in the development and/or maintenance of hypertension[4-6]. A direct attempt to delineate the influence of serotonin on blood pressure in the spontaneously hypertensive rat (SHR) has not yet been made. Since serotonergic neurons are known to have an important role in blood pressure control, there has been considerable value in estimating the turnover rate of serotonin as on index of neuronal activity.

If the efflux of 5-HIAA which is the main metabolite of serotonin was blocked from brain to blood stream by probenecid, the rate of serotonin turnover may be calculated from the initial rise in 5-HIAA level[7].

The action of probenecid has been studied principally in renal tubular[8], isolated choroid plexus[9,10], and ventriculocisternal perfusion preparations[9], where probenecid appears to have a high affinity for the carrier which transport anions across the cellular membrane. In rats, after large doses of probenecid (200 mg/kg, i.p.) the rate of increase in brain 5-HIAA concentrations correlates with the turn-over rate of cerebral serotonin determined by other methods such as the accumulation of serotonin or the initial rate of depletion of 5-HIAA following monoamine oxidase (MAO) inhibition[7].

In the present study, in order to evaluate the relationship between hypertension and central serotonergic neuronal activity, serotonin turnover rates were examined in telencephalon, hypothalamus/thalamus, midbrain, pons/medulla and cerebelluum of 14-week-old SHR with established hypertension and age-matched WKY rat.

MATERIALS AND METHODS

Experimental animals and materials

14-week-old female SHR with established hypertension and age-matched female WKY rats were used.

Before experiment, all rats were acclimated to

our housing facilities for two weeks. Food and water were available *ad libitum*. All experiments were initiated at approximately the same time (10:00 A.M.) each day.

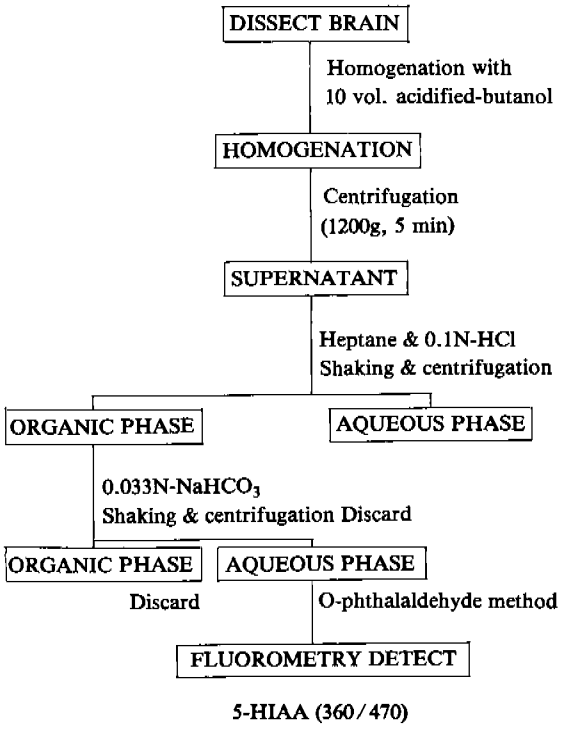
5-hydroxyindole-3-acetic acid was purchased from Sigma Chemical Company (St. Louis, Mo, U.S.A.). O-phthalaldehyde was purchased from TOKYO KASEL (Tokyo, JAPAN).

Probenecid was obtained from the Hanmi Pharmaceutical Company (Seoul, Korea).

All other chemicals were of analytical grade commercially available. Probenecid was dissolved in 1M NaOH and adjusted to PH 7.8 with phosphate buffer.

Experimental design

Experimental design was set to evaluate the differences of central serotonergic neuronal activity between SHR with established hypertension and age-matched WKY rat. Blood pressure was measured in 14-week-old SHR and age-matched normotensive WKY rat. Serotonin turnover rate was measured as an index of serotonergic neuronal activity in 5 brain areas (telencephalon, hypothalamus/thalamus, midbrain, pons/medulla and cerebellum).



Scheme I. 5-HIAA determination method

Brain section

Rats were killed by decapitation and 5 brain regions were dissected using a modification of the method of Miller and Cox[11].

Blood pressure measurement

Systolic blood pressure was calculated by an indirect tail-cuff method of Peffer *et al.*[12] After constant pressure was endowed via occlusion cuff connected to Programmed Electrosphygmomanometer PE-300 (Narco, Biosystem), the blood pressure measured by Korotkoff sounds microphone was recorded in physiograph (Narco Trace™-80). The point at which the first pulse appears was taken as systolic blood pressure. The value for blood pressure represents the mean of the data from at least five measurements in each animal.

Measurement of serotonin turnover rate

Serotonin turnover rate was measured using the method originally described by Neff *et al.*[7] After probenecid (200 mg/kg, i.p.) treatment, serotonin turnover rate was calculated by the 5-HIAA accumulation rate in zero time, 30 min and 60 min.

Determination of 5-HIAA

5-HIAA concentration was determined by the method of Miller and Cox[11]. From the various areas of rat brain homogenized in acidified n-butanol, fluorescence was measured in a spectrofluorimeter (BAIRO-ATOMIC). Activation and emission wavelengths were 360 nm and 470 nm, respectively.

RESULTS

Blood pressure changes in SHR and WKY rat.

Blood pressure changes after probenecid treatment (200 mg/kg, i.p.) were represented in Fig. [6].

In zero time, the blood pressure of normotensive WKY rat was 124 ± 3 mmHg and that of SHR was 186 ± 8 mmHg. This result indicates that the high blood pressure of SHR was fully established. In 60 min after probenecid treatment, blood pressure of SHR was dramatically dropped and in 90 min slowly recovered.

In case of WKY rat, in 60 min after probenecid treatment blood pressure was significantly lowered and in 90 min slowly recovered.

Serotonin turnover rate in telencephalon

The 5-HIAA concentration in telencephalon of SHR measured at zero time was significantly higher

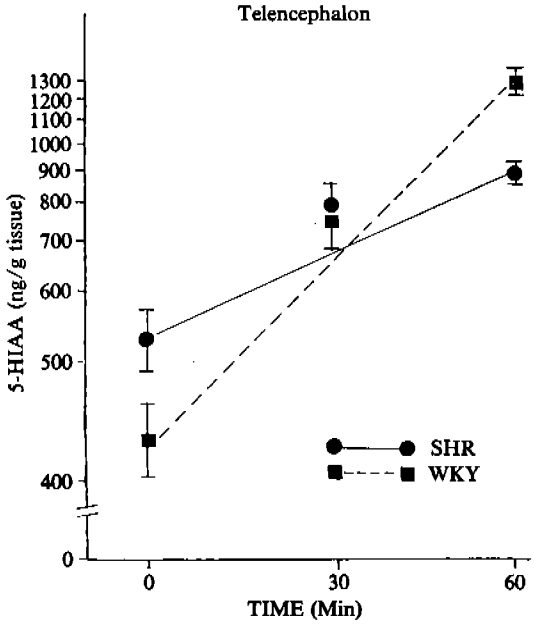


Fig. 1. 5-HIAA concentrations after probenecid treatment (200 mg/kg, i.p.) in telencephalon of SHR and WKY.

Each point represents the mean ± S.E.M. from at least 4 animals.

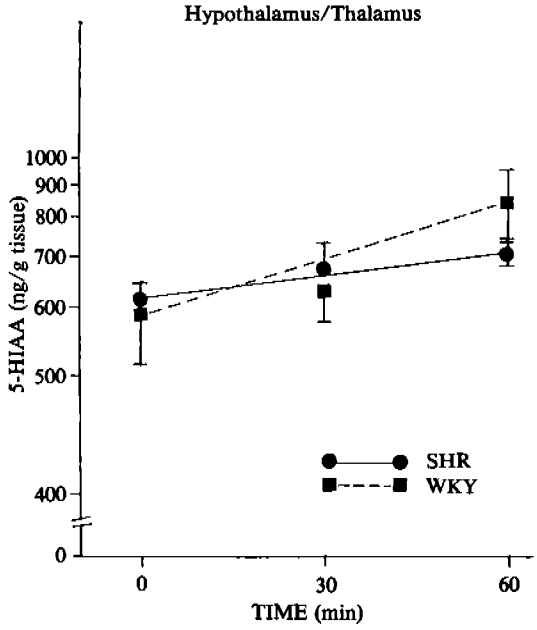


Fig. 2. 5-HIAA concentrations after probenecid treatment (200 mg/kg, i.p.) in hypothalamus/thalamus of SHR and WKY.

Each point represents the mean ± S.E.M. from at least 4 animals.

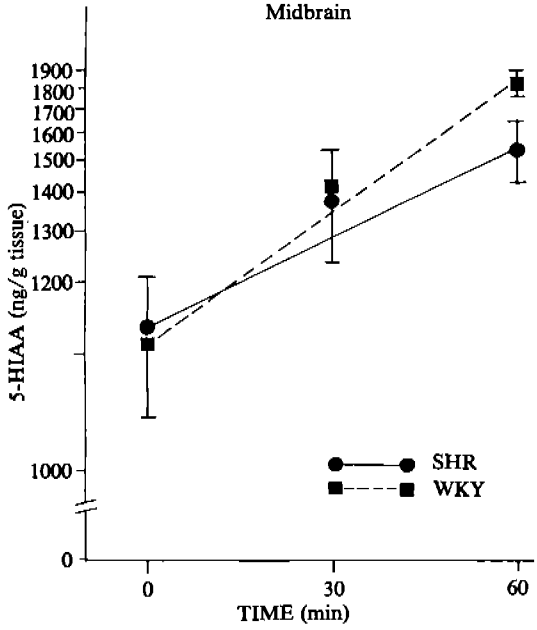


Fig. 3. 5-HIAA concentrations after probenecid treatment (200 mg/kg, i.p.) in midbrain of SHR and WKY.

Each point represents the mean ± S.E.M. from at least 4 animals.

than that of WKY rat as was steady state 5-HIAA concentration obtained from linear regression line (Fig. 1). Rate constant measured from the 5-HIAA accumulation rate in zero time, 30 min and 60 min was significantly lower in SHR than in WKY rat. Serotonin turnover rate obtained from the multiplication of rate constant and steady state 5-HIAA concentration was significantly lower in SHR than in WKY rat.

Serotonin turnover rate in hypothalamus/thalamus

Significant differences between SHR and WKY rat were found niether in 5-HIAA concentration of hypothalamus/thalamus measured at zero time nor in steady state 5-HIAA concentration (Fig. 2). Rate constant measured from the 5-HIAA accumulation rate showed no significantly differences between SHR and WKY rat. But the serotonin turnover was significantly lower in SHR than in WKY rat.

Serotonin turnover rate in midbrain

These were no significant differences between two experimental groups either in 5-HIAA concentration of midbrain measured at zero time or in steady state 5-HIAA concentration.

Rate constant measured from the 5-HIAA ac-

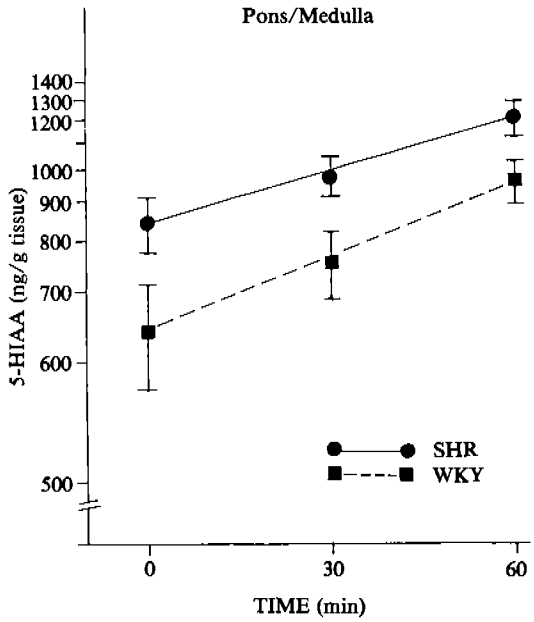


Fig. 4. 5-HIAA concentrations after probenecid treatment (200 mg/kg, i.p.) in pons/medulla of SHR and WKY.

Each point represents the mean ± S.E.M. from at least 4 animals.

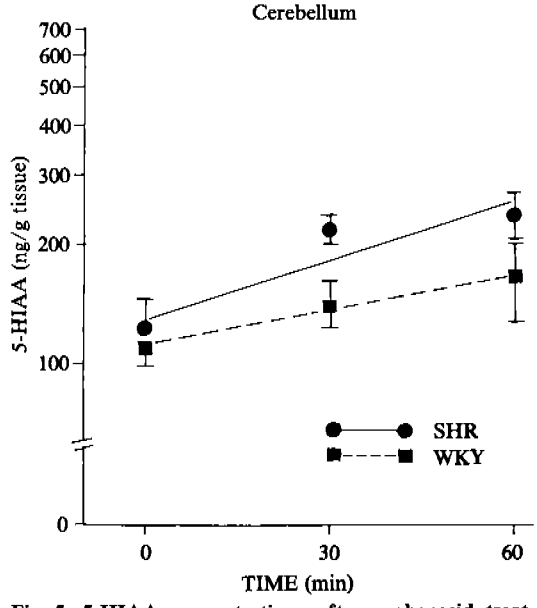


Fig. 5. 5-HIAA concentrations after probenecid treatment (200 mg/kg, i.p.) in cerebellum of SHR and WKY.

Each point represents the mean ± S.E.M. from at least 4 animals.

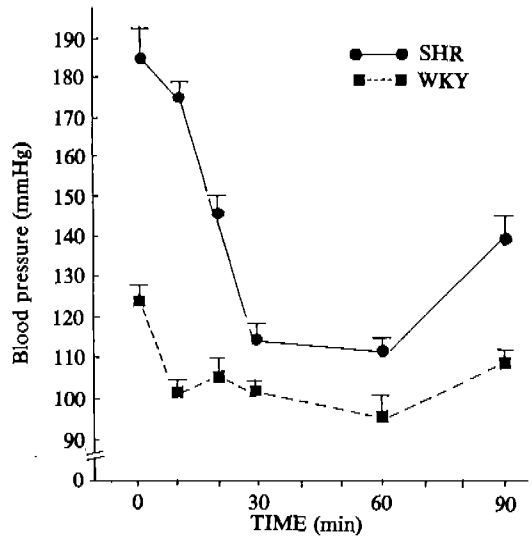


Fig. 6. Blood pressures in SHR and WKY at various time intervals after probenecid administration at zero time.

Each point represents the mean ± S.E.M. from at least 4 animals.

cumulation rate exhibited no significant difference between SHR and WKY rat, But the serotonin turnover rate was significantly lower in SHR than in WKY rat (Fig. 3).

Serotonin turnover rate in pons/medulla

The 5-HIAA concentration in pons/medulla of SHR was significantly higher than that of WKY rat as was steady state 5-HIAA concentration. Rate constant measured from the 5-HIAA accumulation rate exhibited no significant differences between SHR and WKY rat. And there were no significant differences in serotonin turnover rate of pons/medulla between SHR and WKY rat (Fig. 4).

Serotonin turnover rate in cerebellum

Significant differences between two experimental groups were shown neither in 5-HIAA concentration of cerebellum measured at zero time nor in calculated steady state 5-HIAA concentration obtained from linear regression line. Rate constant measured from the 5-HIAA accumulation rate represented no significant differences between SHR and WKY rat. But the serotonin turnover rate was significantly higher in SHR than in WKY rat (Fig. 5).

DISCUSSION

Blood pressure in 14-week-old SHR was 186 ± 8

Table 1. Summary of results

CNS Area	Animals	Observed zero time 5-HIAA (ng/g)	Calculated zero time 5-HIAA (ng/g) (a)	Fractional rate constant (Kt) (1/h)	Turnover (Ktxa) (ng/g/h)
Telencephalon	Control	424±28	425±31	1.110±0.082	471±34
	SHR	526±31*	548±37*	0.536±0.081	294±20**
Hypothalamus/	Control	601±84	563±74	0.345±0.163	194±26
thalamus	SHR	603±37	603±51	0.144±0.124	85± 7**
Midbrain	Control	1129±88	1105±96	0.474±0.112	524±46
	SHR	1130±76	1146±84	0.303±0.073	347±26*
Pons/medulla	Control	639±62	621±73	0.424±0.103	263±31
	SHR	840±50*	824±64*	0.360±0.360	296±23
Cerebellum	Control	110± 6	111±29	0.356± 0176	40±10
	SHR	123±14	131±31	0.661±0.152	87± 9*

Values are shown as mean±S.E.M. from at least 4 animals.
*indicates a significant difference from normotensive control rats.
(*$P<0.05$; **$P<0.01$; ***$P<0.001$)

mmHg and that was significantly higher than that of WKY rats (124±3 mmHg).

In 14-week-old SHR, the hypertension was fully developed. A role of serotonergic neurons in the central regulation of blood pressure has been suggested.

Some evidence supports the idea that enhancement of central serotonergic function lowers blood pressure.

The administration of tryptophan, the serotonin precursor, in normotensive rat produces no conspicuous change in blood pressure, but in SHR that produces significant drop in blood pressure[13,14]. And the serotonin precursor 5-hydroxytryptophan (5-HTP) caused a fall in blood pressure in conscious rats[15], in SHR[16]. in cat[17], and in dog[4,18] found that treatment of conscious normotensive or spontaneously hypertensive rats with single (200 mg/kg, i.p.) or multiple (40 to 250 mg/kg/day for 3 days) doses of PCPA (p-chlorophenylalanine) significantly increased blood pressure by 20 hours after treatment.

The blood pressure remained elevated (approximately 20 mmHg) for at least 4 days, at a time when 5-HT was still significantly depleted from brain.

Recently β-endorphin was reported to lower blood pressure by a mechanism suggested to involve enhanced central serotonin activity[19].

Collectively, these observations suggest that 5-HT neurons normally inhibit transmission in central sympathetic pathways. These results are shown to relate with our experimental results. The abnormally lower turnover rate of serotonin in telencephalon, hypothalamus/thalamus and midbrain may suggest a negative influence of central serotonin neurons on blood pressure. Fuller *et al.*[16] have shown that combined treatment with fluoxetine, a specific inhibitor of the uptake pump on serotonin neuron, and 5-HTP decreased blood pressure in SHR.

Some pharmacologic Studies, however, have yielded opposite conclusions that suppression of central serotonergic function lowers blood pressure and that enhanced serotonin activity increases blood pressure. Lamber *et al.*[20] have shown that infusion of serotonin into the cerebroventricular system produces a pressor effect in rats.

Wolf *et al.*[21] have observed that microinjections of serotonin into either the preoptic region of the anterior hypothalamus (AH/PO) or the nucleus tractus solitarius produces a rise in mean arterial pressure. Antonaccis *et al.*[22] reported that the i.v. or i.c.v. injection of methysergide, a 5-HT antagonist, produced a reduction in blood pressure. And Kuhn *et al.*[23] have reported that the electrical stimulation of 5-HT nuclei (*e.g.*, posterior portions of raphe pallidus and obscuris, anterior portion of raphe magnus and dorsal and median raphe nuclei) elicits pressor responses.

These extremely variable results undoubtedly reflect the complexity of 5-HT neuronal pathways and their interaction with the central sympathetic system

Smith *et al.*[24] measured the serotonin synthesis rate in brain by the accumulation rate of 5-hydroxytryptophan (5-HTP) following decarboxylase inhibition. *In vivo* serotonin synthesis rate in pons/medulla and spinal cord of 4-week-old SHR was higher than that of normotensive rats.

This results suggest that the early change of central serotonergic nervous system may act as a triggering factor in the development of hypertension. In case of hypothalamus in 8-week-old SHR, *in vivo* serotonin synthesis rate shows low tendency than that of normotensive rats. In case of pons/medulla and spinal cord, there are no differences between two animal groups.

As Smith *et al.*[24] regarded 3-week-old rat as in prehypertensive state and 8-week-old rat as in hypertensive state, our results of 14-week-old rat do not show a similar pattern.

It is a difficult problem to conclude that whether the abnormally lower turnover rate of serotonin in telencephalon, hypothalamus/thalamus, and midbrain of SHR may act an important role in hypertension maintenance mechanism or may show as a result of a reflex in the process of rapid hypertension formation. According to the recent research, it is suggested that at least three types of 5-HP receptors exist in central nervous system.

The first type of 5-HT receptor mediates on inhibition of neuronal activity in areas such as the suprachiasmatic nucleus, the ventrolateral geniculate and the cortical and basolateral geniculate and the cortical and basoloteral nuclei of amygdala[25].

Second type of postsynaptic 5-HT receptor has been shown to facilitate excitatory input to motor nuclei[26,27].

Finally, third type of 5-HT receptor mediates a 5-HT-induced inhibition of serotonergic neurons in the dorsal raphe nucleus (*i.e.*, a presynaptic or autoreceptor)[28].

This hypothesis may be related to our experimental results.

It can be suggested that the abnormally lower trunover rate of serotonin in telencephalon, hypothalamus/thalamus and midbraim of SHR than that of WKY rat may act an important role in the maintenance of hypertension in SHR by the mechanism of negative effect on inhibited neuronal activity.

Alterations in the dynamics of the cerebral 5-HT system can alter blood pressure regulation but unfortunately, statements concerning the role of 5-HT in BP regulation can only be made with caution at this time.

Control of blood pressure is obviously a very complex process, and any understanding role of that a single factor such as 5-HT may play in blood pressure regulation is complicated by the adaptibility of the organism under study and by the extent to which the control of blood pressure is buffered.

There is little doubt that 5-HT system is just one of many regulatory neuronal networks whose role in blood pressure regulation can best be understood when viewed in concert with other dynamic integrating central and peripheral control systems.

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